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(54) Title: METHOD FOR CHEMICAL PROMOTION OF THE EFFECTS OF LOW CURRENT TRANSCRANIAL ELECTROSTIMULATION

(57) Abstract

Method of providing relief from painful or stressful stimuli, or remediating imbalances or deficiencies in the neuroactive substances that modulate neurohumoral mechanisms, which involves concomitant administration of a neuroactive chemical promoter and transcranial electrostimulation. This combination enhances the ability of the central nervous system to provide relief from, for instance, pain, addiction withdrawal, anxiety and depression.

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DESIGNATIONS OF "DE"

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METHOD FOR CHEMICAL PROMOTION OF THE EFFECTS OF LOW CURRENT, TRANSCRANIAL ELECTROSTIMULATION

INTRODUCTION

The present invention relates to the concomitant administration of transcranial electrostimulation and chemical promotors to produce greater effects when acting together then either would produce acting individually. In more detail, the present invention relates to a method of medical treatment of a mammal which produces greater and longer lasting analgesic, anti-abstinence, anti-withdrawal, anti-anoxiant, anxiolytic, anti-psychotic, anti-depressant, relaxant, and anti-inflammatory effects than can be produced with drugs associated with these effects with the use of the same, or even lesser, dosages needed to achieve the effect.

Transmission of nerve impulses between neurons is an electrochemical process in which electrical impulses reaching the neuron's terminal cause various chemicals, referred to as neurotransmitters, to be emitted. Once released by the neuron, the neurotransmitter crosses the synapse (a gap of about 20 nm) to the membrane of the next neuron in the neuronal circuit. There, the neurotransmitter interacts with specific receptors and generates a new electrical impulse, which continues the signal.

Alternatively, the chemical can be released into the general stream of extracellular fluid and subsequently join the flow of cerebrospinal fluid (CFS). The released chemical may simply interact locally with receptors on neurons in the immediate neighborhood (paracine action) or the chemical may travel further to interact with specific receptors positioned at relatively distant target sites, in adjacent brain regions or in other parts of the body (endocrine action).

The released chemicals mostly act simply as neurotransmitters, but a subcategory (which may be co-released with the neurotransmitter) can also act as

modulating agents (neuromodulators). These agents
influence the extent to which their own, or adjacent,
nerve terminals can either release, or respond to the
action of, neurotransmitters. Released chemicals which
travel to and act at other parts of the body are
generally referred to as hormones.

Neuromodulation is one of the most important
intrinsic properties of individual neurons.
Neuromodulation is generally defined as the ability of
neurons to alter their electrical properties in response
to intracellular biochemical changes resulting from
synaptic or hormonal stimulation. This property not
only allows the nervous system to adapt its control of
physiological functions to a continually changing
environment, but also forms the basis for many
long-lasting changes in human behavior and in the
behavior of animals. Changes in behavior that can be
related directly to changes in the electrical responses
of specific neurons include the triggering of
long-lasting, but relatively fixed and inate, behaviors
such as feeding and reproduction, as well as alterations
in behaviors that can be ascribed to learning.

It is these chemicals, and their effects on behav-
iors and nervous system functions, with which the
present invention is concerned. Specifically, the
present invention is concerned with the interaction of
these chemicals with the effects of transcranial
electrostimulation on the behaviors and the function of
the nervous system. Transcranial electrostimulation, or
TE, is described in detail in U.S. Patent No. 4,646,744,
and that description is incorporated herein in its
entirety by this specific reference to that patent. In
brief, TE may be described as the application of
extremely low (on the order of 10 μA), pulsed,
charge-balanced current across the head through
electrodes of opposite polarity attached to low
impedance sites on the head. The current levels applied

are well below the current amplitudes commonly employed
in electroacupuncture, transcutaneous electrical nerve
stimulation (TENS), and other electrostimulation
techniques, and the waveform used is significantly
different. Nevertheless, a growing body of scientific
literature documents the effectiveness of TE in, for
instance, inducing analgesia (Skolnick, M.H., et al.,
"Low current electrostimulation produces
naloxone-reversible analgesia in rats," 13 Soc.
Neurosci. Abstr. 1304 (1987)) and reducing the severity
of opiate withdrawal syndrome (Murray, J.B., et al.,
"Transcranial auricular electrostimulation (TCET):
Naloxone reversible attenuation of pain sensitivity and
opiate abstinence syndrome," 13 Soc. Neurosci. Abstr.
1304 (1987)). These same references document the
reversibility of these effects with exogenous naloxone,
a potent inhibitor of the effects of endogenous opiates
which exerts its effect by blocking the opiate recep-
tors, suggesting strongly that TE effects are mediated
by release of endogenous opioids. The references also
provide evidence to indicate that TE is as potent as
inductor of analgesia as focal (in brain electrodes)
electrical stimulation (Dong, W. P., et al., "Effects of
dorsal raphe, habenula and external stimulation on
septal neurons in the rat", 14 Proc. Soc. Neurosci. 855
(1988)).

SUMMARY OF THE INVENTION

Surprisingly, however, and as will be shown by the
experimental evidence set out below, it has been dis-
covered that when various neuroactive chemical promotors
are administered concomitantly with TE, a more intense
and prolonged physiological result is achieved than was
expected. In one aspect, therefore, the present
invention is characterized broadly as a method of
medical treatment of a mammal involving the enhancing of
the normal efforts of the central nervous system to

ameliorate or otherwise compensate for such stress as
pain, anxiety, addiction withdrawal symptoms,
depression, and inflammation, comprising the concomitant
administration of a neuroactive chemical promotor and
subsensory, transcranial electrostimulation.

This medical treatment method is also useful in
remediating imbalances or deficiences in levels of
neuroactive substances that modulate neurohumoral
mechanisms in living beings. Concomitant administration
of TE and a neuroactive chemical promoter provides a
treatment modality which can replenish, initiate or
invigorate production of and/or compensate for, such
deficiencies and/or imbalances.

In another aspect, the present invention is
characterized as a kit for providing medical treatment
of a mammal, which kit comprises a neuroactive chemical
promotor in a form suitable for administration to a
mammal and means for concomitant administration of
transcranial electrostimulation to the mammal. In a
third aspect, the present invention contemplates the use
of a neuroactive chemical promoter for the manufacture
of a medicament for administration to a mammal to which
transcranial electrostimulation is concomitantly
administered. In another apsect, the present invention
is characterized as a pharmaceutical composition for
providing medical treatment of a mammal in combination
with transcranial electrostimulation, comprising as an
active ingredient a neuroactive chemical promotor.

Such kits, compositions, and methods are
characterized by a number of advantages over previously
available techniques. The ability to achieve analgesia
to relieve chronic pain, to assist relaxation in
combatting stress related disorders and to ameliorate
drug withdrawal syndromes with a non-addictive modality
for which tolerance does not increase is of obvious
clinical significance. Further, the same effects can be
achieved as are achieved by the therapeutic use of many

specialized drugs, but with lower dosage levels or, in
some cases, without even administering the drug (e.g.,
by accomplishing the same effect by using a different
agent), therefore potentially decreasing the cost and/or
side effects of drug therapy. Another significant
advantage is that the ameliorative effects are not only
more pronounced but of longer duration than the effects
produced by administration of the drug alone. Such
results can even be achieved using subclinical doses of
the neuroactive chemical promotor of the present
invention.

It is, therefore, an object of the present in-
vention to provide a medical treatment method for
mammals for use in, for instance:

(1)   smoking cessation,

(2)   withdrawal syndrome from addiction of, for
instance, morphine, heroin and related narcotics, as
well as other addictive drugs such as alcohol and
cocaine,

(3)   chronic pain resulting from, for instance,
arthritis, migraine headaches, low back pain, failed
surgery, terminal cancer, and other sources,

(4)   acute pain,

(5)   psychoses,

(6)   depression

(7)   anxiety,

(8)   thermal insult, and

(9)   any of a host of stress-related disorders such
as insomnia, certain forms of obesity and anorexia,
post-traumatic stress disorder, and clinical depression.

Another object of the present invention is to
provide a method of treating mammals for stressful
stimuli such as those listed in the preceding paragraph
which promotes or enhances the normal mechanisms by
which the central nervous system ameliorates the effects
of such stimuli.

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Another object of the present invention is to
provide an effective method for treating mammals
experiencing stressful stimuli such as those listed
above with the same or reduced dosages as currently used
to achieve enhanced and/or prolonged effects.

Other objects of the present invention will be made
clear to those skilled in the art by the following
detailed description of several preferred protocols for
practicing the invention. In setting out these
descriptions, it is not intended that the invention be
limited to these specific embodiments.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a bar graph showing the decreased
TE-induced analgesia resulting from administration of
p-chlorophenylalanine (pCPA), which depletes serotonin
levels, in rats as described in Example 3, below.

Figure 2 is a bar graph showing the restoration of
TE-induced analgesia after restoring serotonin levels to
pCPA-treated rats by administering exogenous serotonin
as described in Example 3, below.

Figure 3 is a bar graph showing the enhancement of
TE-induced analgesia in rats by concomitant
administration of thiorphan as described in Example 4,
below.

Figure 4 is a bar graph showing the enhancement of
TE-induced analgesia in rats by concomitant
administration of acetorphan as described in Example 5,
below.

Figure 5 is a bar graph showing the effect of
concomitant administration of proglumide and TE in
morphine addicted rats upon deprivation of morphine as
described in Example 13, below.
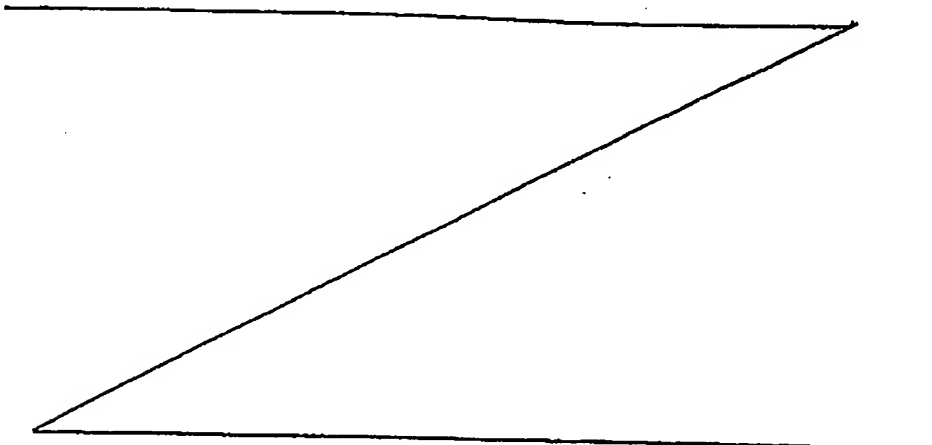

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The reaction to and management of pain, stress and
anxiety are modulated by a host of neurotransmitters

synthesized and pharmacologically active in the
mammalian brain and spinal chord. These endogenous
transmitters are synthesized in various regions of the
brain from various chemical precursors and are broken
down or metabolized by enzyme systems at various sites
in the central nervous system (CNS). Neurotransmitters
have been shown to induce or be involved with analgesic,
anxiolytic, sedative, hypnotic, CNS depressant, and
psychotropic effects.

A variety of chemical agents have been discovered
which interact with neurotransmitter systems. These
agents may, for instance, possess a chemical structure
which mimics a particular transmitter or class of
transmitters and which binds to the cellular receptors
uniquely associated with the transmitter family. For
example, exogenous heroin or morphine cross the
blood-brain barrier and displace the brain's endogenous
peptidyl opiates, the endorphins and enkephalins.

These chemical agents, referred to generally herein
as neuroactive chemical promoters, include any sub-
stances which are capable of exerting an effect on the
CNS which enhances the capacity of the CNS to relieve or
ameliorate the effects of such stressful stimuli as
pain, addiction withdrawal syndrome, and so on as listed
above, or which are benefical in remediating imbalances

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or deficiencies in the endogeneous neuroactive
substances that modulate neurohumoral mechanisms. Such
substances may include, but are not limited to,

 endorphins such as β-liptropin, adrenocortico-
  tropin, alpha-melanocyte-stimulating hormone,
  met-enkephalin, leu-enkephalin, peptides E and
  F, metorphamide, amidorphin, dynorphin,
  dynorphin B, and alpha-neo-endorphin,

 precursors of various endorphins such as
  pro-opiomelanocortin, pro-enkephalin A and
  pro-enkephalin B (pro-dynorphin),

 enzymes active in the metabolic pathways involved
  in production or degradation of the above-
  listed endorphins and enkephalins such as
  endorphinase, enkephalinase, and
  carboxypeptidases,

 inhibitors of the enzymes active in the metabolic
  pathways involved in production or degradation
  of endorphins such as acetorphan, thiorphan
  and kelatorphin, a D-form amino acid such as
  D-alanine, D-arginine, D-asparagine,
  D-aspartic acid, D-cysteine, D-glutamine,
  D-glutamic acid, D-glycine, D-histidine,
  D-isoleucine, D-leucine, D-lysine,
  D-methionine, D-phenylalanine, D-proline,
  D-serine, D-threonine, D-tryptophan,
  D-tyrosine, and D-valine, or a di- or
  tri-peptide comprised of any one or more of
  these D-form amino acids, amino acid
  derivatives such as 3-aminotyrosine, a thiol
  benzyl amino acid such as (2-[mercapto-3-
  phenyl-propanol]-L-leucine or any N-[(R,S)-
  2-carbethoxy-3-phenyl-propanol]-L-leucine, a
  carboxyalkyl methylester, hydrocinnamic acid
  or other propionic acid derivative such as
  ibuprofen, naproxen, and piroxicam, a barbitu-
  ate such as secobarbital, amobarbital, and
  pentobarbital, pyrophosphate, O-phenanthro-

line, phosphamidon, Z-leucine-NHOH, Z-glycine-
    NHOH or an antibiotic such as bacitracin,
    bestatin, or puromycin,
indoleamines such as serotonin, tryptophan, trypta-
    mine, histamine, melatonin, indoleacetic acid,
    5-hydroxytryptophan, (N')-methyl and (N')-
    acetylserotonin, N,N-dimethyl tryptamine
    (DMT), bufotenine, psilocine, psilocybin, and
    other derivatives of tryptamine,
enzymes active in the metabolic pathways involved
    in production and degradation of indoleamines
    such as 5-hydroxytryptophan decarboxylase,
    tryptophan 5-monoxygenase, monoamine oxidase,
    and tryptophan hydroxylase,
inhibitors of the enzymes active in the metabolic
    pathways involved in production and degrada-
    tion of indoleamines such as p-chlorophenylala-
    nine,
5-hydroxytryptophan antagonists such as the ergot
    alkaloids such as lysergic acid diethylamide
    (LSD) and methysergide (generally in the form
    of methysergide maleate), H_1 blockers of the
    ethylenediamine type, various indole
    compounds, spiroperidol, pirenperone,
    cyproheptadine, phenothizaines such as
    chlorpromazine, β-haloalkylamines such as
    phenoxybenzamine, siperone, metergoline,
    metitepine, mianserin, pizotyline (pizotifen),
    cinanserin, and ketanserin, and substances
    such as clomipramine and fluoxetine which
    inhibit uptake,
catecholamines such as noradrenalin, adrenalin,
    dopamine, 3,4-dihydroxyphenylalanine (dopa),
    normetanephrine and metanephrine,
enzymes active in the metabolic pathways involved
    in production or degradation of catecholamines
    such as monoamine oxidase, catechol-O-methyl-

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transferase, dopamine β-hydroxylase, phenyl-
ethanolamine-N-methyltransferase,   and
tyrosine-3-monooxygenase,
inhibitors of the enzymes active in the metabolic
    pathways involved in production and degrada-
    tion of catecholamines such as pargyline,
    nialamide, and tetrahydrobiopterin,
catecholamine agonists and antagonists such as the
    phenothiazones, thioxanthenes, and buty-
    rophenones, isoproterenol, prazosin,
    rauwolscine, yohimbine, metoprolol, proctolol,
    butoxamine, cocaine, imipramine and other
    tricyclic antidepressants, guanethidine,
    bretylium, sympathomimetic drugs such as
    tyramine and ephedrine, and agents which
    inhibit catecholamine storage such as
    reserpine, tetrabenazine and α-methyl-m-
    tyrosine
amino acids present in the central nervous system
    such as gamma-aminobutyrate (GABA), glycine,
    glutamate and aspartate and those amino acids
    capable of inducing central nervous system
    response such as β-alanine and taurine,
enzymes active in the metabolic pathways involved
    in production and degradation of these amino
    acids such as glutamate decarboxylase,
amino acid antagonists and/or binding competitors
    such as bicuculline, picrotoxin, apiamine,
    strychnine, glutamate diethylester, alpha-
    amino adipate, 2-amino-5-phosphonovalerate and
    lactonized kainate, agents that mimic the
    action of these amino acids such as muscimol
    or inhibit the re-uptake of the amino acid
    such as 2,4-diaminobutyrate, nipecotic acid,
    guvacine, and 2-hydroxy-γ-aminobutyrate,
    agents which alter the rate of synthesis or
    degradation of the amino acid such as

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aminooxyacetic acid or which potentiate the
response to the amino acid such as the
benzodiazepines diazepam, clordiazepocide,
clorazepate, and flurazepam,

a variety of peptides either found in the central
nervous system or which are capable of exert-
ing various effects on the CNS including
carnosine, homocarnosine, anserine,
pyroglutamylhistidylprolinamide, adreno-
corticotropin hormone (ACTH), chole-
cystokinin, somatostatin, substance P,
scotophobin, gastrin, vasopressin, oxytocin,
vasoactive intestinal peptide (VIP), glucagon,
thyrotropin releasing hormone, and secretin,

enzymes active in the metabolic pathways involved
in production and degradation of the above-
listed peptides, such as carnosine synthetase,

inhibitors of the enzymes active in the metabolic
pathways involved in production and degrada-
tion of the above-listed peptides, and

antagonists of the above-listed peptides, e.g.,
cholecystokinin, such as proglumide and
lorglumide,

cholinergic agents such as acetylcholine,

enzymes active in the metobolic pathways involved
in production and degradation of cholinergic
agents such as acetylcholinesterase, choline
acetyltransterase or butyrylcholinesterase,

antagonists of the enzymes active in the metabolic
pathways involved in production and
degradation of cholinergic agents and
substances which block the uptake of such
agents such as methacholine, nicotine,
physostigmine, or diisopropyl
phosphorofluoridate.

In addition to the substances listed above, the phrase
"neuroactive chemical promoter" is intended to include a
large number of substances which are not necessarily

easily classified into one of the above groupings but which nonetheless are capable of exerting an effect on the central nervous system which enhances the capacity of CNS to relieve or ameliorate the effects of, for instance, the above-listed stressful stimuli. Such substances can best be described by citation to a reference characterized the substance in detail as follows:

pyrido[2,3-e]-as-triazine derivatives as described in U.S. Patent No. 4,324,786, Messmer,

heteramino benzofurans as described in U.S. Patent No. 4,451,462, Wenk,

isochromans as described in U.S. Patent No. 4,487,774, McCall,

4,5 α epoxymorphinan-6-spiro-2'-(4'-carboxy,1',3'-thiazolindine) derivatives as described in U.S. Patent No. 4,496,570, Bodor,

pyrazolo/4,3-C/pyridines as described in U.S. Patent No. 4,500,525, Winters,

imidazo[1,2-A]pyridine derivatives as described in U.S. Patent No. 4,501,745, Kaplan, et al.,

2-benzoxepins as described in U.S. Patent No. 4,556,656, McCall,

pyrazolo[1,5-a]pyrimidines as described in U.S. Patent No. 4,576,943, Tomcufcik, et al.,

bridged pyridines as described in U.S. Patent No. 4,587,253, Halczenko, et al.,

condensed pyrrolinone derivatives as described in U.S. Patent No. 4,590,189, Hiraga, et al.,

6-fluoro-3-[3-(1-heterocyclo)-propyl]-1,2-benz-isoxazoles as described in U.S. Patent No. 4,591,586, Davis, et al.,

8-[3-(4-amino carbonyl piperazino and piperidino propyl}xanthenes as described in U.S. Patent No. 4,599,338, Regnier, et al.,

substituted and bridged pyridines as described in
U.S. Patent No. 4,599,341, Halczenko, et al.,
2-(arylalkyloxymethyl) morpholines as described in
U.S. Patent No. 4,605,654, Cousse, et al.,
benzofuran- and benzopyran-carboxamide derivatives
as described in U.S. Patent No. 4,617,714, Tahara, et
al.,
heterocyclic derivatives bearing an amino radical
as described in U.S. Patent No. 4,647,557, Moinet, et
al.,
3-amino-2, 3-dihydro-1-benzoxepines as described
in U.S. Patent No. 4,672,062, Ohlendorf, et al.,
fused imidazoheterocyclic compounds as described in
U.S. Patent No. 4,772,600, Tomczuk, et al.,
3, 6-disubstituted trizolo [3,4-A] phthalazine
derivates as described in U.S. Patent No. 4,783,461,
Occelli, et al.,
1, 3-dithiolano- 1, 4-dithiino- and 1,
4-dithiepino [2,3-C] pyrrole derivatives as described
in U.S. Patent No. 4,788,191, Hiraga et al., and
1,2,3,3A,8,8A-hexahydro-3A, 8 (and) 1,3A,8-di (and
tri) methylpyrrolo (2,3-B) indoles as described in U.S.
Patent No. 4,791,107, Hamer, et al.
In spite of the length of the above list of patents, it
is not intended that the present invention be limited to
the substances described therein. There are many more
substances included within the scope of the phrase
neuroactive chemical promotors; however, the invention need
only be exemplified.
As noted above, an appropriate apparatus for admini-
stering transcranial electrostimulation (TE) is described in
U.S. Patent No. 4,646,744, incorporated herein by reference.
However, the phrase "means for administration of trans-
cranial electrostimulation" is used throughout this speci-
fication because it is not intended that the apparatus be

limited only to that which is set out in that patent.
Actual experimental protocols are set out below, but in
general, TE can be described as the application of a
continuous series of sub-sensory electrical pulses from
one side of the patient's head to the other through
electrodes of opposite polarity affixed to the patient's
head. Extensive experimentation has shown that those
sites on the head having the relatively lowest impedance
are the best sites for placement of the electrodes in
that they are more effective in inducing a response.
Further, the impedance must be low enough in an absolute
sense to make it possible to achieve the desired current
level (see below) at a voltage which is below the
threshold of detection by the patient. In both humans
and laboratory animals, it is particularly preferred
that the electrodes be affixed to the external ears, or
pinnae; however, the mastoid process has also been used
to advantage on human patients. On the ear, as is the
case for other locations on the head, a change in
position of as little as two millimeters can make
significant differences in impedance; preferred sites in
humans are the ear lobe, inner ear and upper ear, on
rats, the base of the ear and the apex of the antihelix
are preferred.

Experimentation has also shown that stimulus
sessions of about 30 minutes in small animals such as
rats and 45 minutes in humans produce effects which
persist for three or more hours. The optimum frequency
is approximately 10 Hz, and the optimum current is in
the range of about 5 to about 25 μA, and in humans, from
about 10 μA to about 40 μA. Further, the results are
generally improved when biphasic waveforms are used to
deliver a charge-balanced current rather than mono-
phasic, non-charge balanced waveforms. The phrase
"biphasic, charge-balanced waveform" refers to a series
of generally rectangular pulses having, for instance,
approximately a 10 μA, 2.0 msec positive phase separated
by 98.0 msec negative phases having an amplitude of

approximately 0.2 µA. It is not intended, however, that
the method of the present invention be practiced only
using those parameters. Satisfactory results can be
obtained by varying these parameters as follows:

pulse width (duration)	about 0.1	– about 8.0 msec
frequency	about 5	– about 50 Hz
current	about 5	– about 40 µA
duration	about 10	– about 60 min.

Further, monophasic, non-charge balanced waveforms,
although not as efficacious as biphasic charge balanced
waveforms, may also be used to advantage by varying the
parameters within these same ranges.

The method of the present invention may be better
understood by reference to the following examples of
several presently preferred embodiments thereof.

EXAMPLE 1: Effect of Amplitude, Frequency, Duration
and Repeated Stimulation

Three hundred and thirty four male Sprague-Dawley
rats weighing between 180 and 210 grams were used. The
animals were housed in groups of four with ad lib food
and water and a 12 hour alternating light/dark cycle.
All animals were naive in the sense that they had no
previous experience with TE; however, a day prior to
each experiment, the subjects were placed in restraints
for one hour to acclimate them to the experimental
conditions.

Animals were anesthetized by injection of 400 mg/kg
chloral hydrate i.p. Gold plated stainless steel
electrodes (cosmeticians' ear piercing studs) were
inserted bilaterally into the pinnae at the apex of the
antihelix. This site was chosen because it resulted in
a relatively lower ear to ear impedance of 8.0 ± 0.4
K ohms (n = 26) as measured with a Grass impedance
meter.

A computer controlled stimulator was used to
produce a continuous series of biphasic, charge
balanced, rectangular pulses which, unless otherwise
specified, had a repetition rate or 10 Hz, first phase

amplitude of 10 μA, and first phase duration of 0.1
msec. The second phase duration was approximately equal
to the interval between consecutive first phases, and
the amplitude such that the net charge delivered in any
cycle was zero. An essentially constant current was
maintained by the stimulator during each phase, irre-
spective of variations in the rat impedance.

Fifteen minutes prior to stimulation, the rats were
placed in adjustable cylindrical plastic restrainers.
Leads with a 200 K ohm resistor in series were connected
to the electrodes, with the positive lead (that deliver-
ing a positive first phase) attached to the animals'
right ears. Each experiment included a "sham control
group" consisting of rats which were implanted, re-
strained and connected as above, but which did not
receive any electrical stimulation.

All observations were carried out by operators who
were blind to the stimulus parameters and to the
identity of sham treated animals. Care was taken to
exclude systematic influences on the arising from
stimulation at different times of day by treating and
testing equal numbers of animals from each stimulation
or sham group in each stimulation session.

Analgesia was assessed using the wet tail flick
method described in Mitchell, D. and R.F. Hellon,
"Neuronal and behavioral response in rats during noxious
stimulation of the tail," Proc. Royal Soc. (Lond.)
169-194 (1977). Immediately preceding and following
electrostimulation, the terminal one inch of each rat's
tail was immersed in water at 50°C. The time in seconds
measured with a stopwatch from submergence to the first
flicking response was taken as the tail flick latency.
If a rat did not respond within 20 seconds, its tail was
removed from the water to prevent tissue damage. The
baseline (pre-stimulation) tail flick latency estab-
lished by using a 50°C water bath was 6.39 ± 0.07
seconds (n = 292). Four successive latencies were de-
termined during both the pre- and post-test; in each

case, the first measurement was discarded and the last
three were averaged to yield pre- and post-stimulation
scores. Each rat's analgesia rating was its mean
post-test latency minus its mean pre-test latency.
Results may be summarized as follows.

One-way analysis of variance indicates a signifi-
cant effect of amplitude on change in tail flick latency
(measured at 30 mins. of 10 Hz, 0.1 msec pulse width TE
at 0, 5, 7.5, 10, 12.5, 15, and 20 µA), $F(6,161) = 2.32$,
$p < 0.02$, according to Dunnett's Test for multiple
comparisons with a single control group.

The results of the experiment varying the duration
of electrostimulation indicate that thirty minutes (as
compared 1, 10, 20, 40, 50 and 60 minutes with 0.1 msec,
10 Hz, 10 µA TE) of TE resulted in the greatest increase
in tail flick latency (1.92 ± 0.20 seconds). One way
analysis of variance reveals a significant effect of
stimulation duration on change in tail flick latency,
$F(6,63) = 3.43$, $p < 0.005$. Dunnett's test for multiple
comparisons with a single control group indicated that
only the group receiving 30 minutes of TE differed
significantly from the sham treated controls, $p < 0.05$.
The group receiving 50 minutes of stimulation closely
approached a significant difference from controls, $p < 0.10$.

Upon measuring the duration of analgesia, it was
found that the electrostimulated rats show greater
latency increases from pretest than sham treated con-
trols at all times tested (0, 40, 80, 120, 160 and 200
mins. after 30 minutes of sham or 0.1 msec, 10 Hz, 10 µA
TE) reaching a peak 40 minutes after the end of stimu-
lation. Two way analysis of variance with one repeated
measures variable reveals a significant effect of TE
(stimulation vs. sham), $F(1,22) = 32.30$, $p < 0.01$, and a
significant, $F(5,110) = 2.32$ (ns), indicating that
differences between treated and sham groups did not
change significantly over 200 minutes. Post hoc compar-
isons using Tukey's HSD test reveal significant differ-

ences between stimulated and sham groups at every time interval. At 120 and 160 minutes post stimulation, the difference between groups was significant at the $p < 0.05$ level. At all other intervals, the difference between groups was significant at the $p < 0.01$ level. The TE treated animals had significantly higher latency increases over pretest at 40 minutes, $p < 0.05$, than at 80, 120, 160 or 200 minutes. Latency increases were significantly higher, $p < 0.05$, immediately following TE than at 120, 160 or 200 minutes after stimulation.

The effects of repeated daily electrostimulation were investigated by analysis of variance with one repeated measured variable, which revealed a highly significant effect of TE (stimulation vs. sham), $F(1,28) = 94.29$, $p < 0.001$, between rats receiving five daily 30 min. sham sessions and five daily 30 minute sessions of 0.1 msec, 10 Hz, 10 μA TE. The time effect (days one through five) was not significant, $F(4,112) = 1.45$ (ns). The interaction effect (TE x days) was also not significant, $F(4,112) = 1.81$ (ns), indicating that the effect of electrostimulation vs. sham did not change significantly over five days. Post hoc comparisons using Tukey's HSD test revealed significant differences, $p < 0.05$ between stimulated and sham animals on each day except the second.

EXAMPLE 2: Effect of Pulse Width, Frequency, Polarity Charge Balance and Monolateral Stimulation

Mice were kept and subjected to the same pre-investigational regimens as described in Example 1 (N = 189). For monolateral experiments, the studs were inserted into either the right or left ear only. An essentially constant (± 2%) stimulus current was maintained by connecting the constant voltage waveform generator in series with a 200 K ohm resistor.

Experiment 1 tested the analgesic effects of different stimulus pulse widths in 63 rats not previously exposed to TE that were randomly assigned to

seven groups of 9 rats each. These groups had 30 minutes of 10 μA, 10 Hz stimulation with, respectively, 0 (sham), 0.1, 0.5, 1, 2, 4 and 8 msec first phase pulse widths. As in all the following experiments, tail flick latencies (TFLs) were determined before and after stimulus by an operator who was blind to the stimulus parameters, and the time difference in seconds determined the animals' analgesia scores.

Experiment 2 tested the analgesic effects of different stimulus frequencies in 63 rats were randomly assigned to seven groups of 9 rats each. These groups had 30 minutes of 10 μA, 2 msec pulse width stimulation at 0 (sham), 5, 7.5, 10.0, 15, 20, and 50 Hz.

Experiment 3 tested the necessity of using a charge balanced stimulus waveform in 27 rats randomly assigned to 3 groups of 9 rats each. These groups had 30 minutes of stimulus with, respectively, no stimulus waveform (sham); 10 Hz, 2 msec, 10 μA stimulation with a charge balanced waveform; or 10 Hz, 2 msec, 10 μA stimulation with a monophasic (non charge-balanced) waveform.

Experiment 4 tested the effect of stimulus polarity on the analgesia produced by TE in 15 rats tested three times in a blind, triple crossover design with 30-minute stimulus sessions. Each rat received a session of sham stimulation; one of 10 Hz, 2 msec, 10 μA charge balanced TE with standard polarity (positive lead on the right ear); and one of 10 Hz, 2 msec, 10 μA TE with reversed polarity (positive lead on the left ear). TFL analgesia scores in this experiment were determined in all animals before and after each session. Statistically weak results led to a repeat of the experiment using a further 12 subjects in the sample triple crossover design.

Experiment 5 investigated the efficacy of several electrode positioning schemes in 36 rats randomly assigned to three groups of 12 rats each. One group had one electrode in each ear, the other two groups had two electrodes in either the left or the right ear as

described. Each animal had 30 minutes of 10 Hz, 2 msec,
10 µA charge balanced TE. TFL analgesia scores were
determined in the usual way under blind conditions.

A one-way analysis of variance with the data from
Experiment 1 indicates a significant effect for pulse
width on the change in tail flick latency, $F(6,56) =$
2.283, $p < 0.05$. According to Dunnett's test for
multiple comparisons with a single control, three groups
(1, 2, and 8 msec pulse widths) differed significantly
from the sham group in post hoc comparison, $p < 0.05$.
Although the 2 msec group showed the greatest overall
degree of analgesia, the difference between this group
and the 1 or 8 msec groups was not statistically signifi-
cant.

The results of Experiment 2, varying frequency of
electrostimulation, indicated that the 10 Hz group
showed the greatest increase, 45%, in tail flick latency
(2.28 ± 0.61 sec). A one-way analysis of variance
reveals a significant effect of electrostimulation
frequency on the change in tail flick latency, $F(6,56) =$
4.012, $p < 0.002$. Post hoc comparisons using Tukey's
HSD test show that only the 10 Hz group differed
significantly from the sham controls, $p < 0.05$.

The results of Experiment 3, effect of charge
balance on changes in tail flick latency, demonstrate
that the greatest increase in latency (2.95 ± 0.43
sec), which represents a 49% increase in response time,
occurred in the rats given charge balanced stimulation.
A one-way analysis of variance shows a significant
effect for both mono- and bipolar stimulation on the
change in tail flick latency, $F(2,24) = 13.703$, $p <$
0.001. Post hoc comparisons using Tukey's HSD test
indicate that both stimulus groups, regardless of charge
compensation, were significantly different from sham,
$p < 0.01$. Although the biphasically stimulated group
showed a TFL increase averaging 1 sec more than the
monophasically stimulated group, this difference was not
statistically significant.

Experiment 4 was initially conducted with a group
of 15 animals, and a one-way analysis of variance
indicated a significant effect of stimulation, $F_{(2,28)}$ =
4.579, $p < 0.02$. Comparison with Tukey's HSD test
revealed that the group receiving positive stimulation
on the right ear had a significant increase in TFL
compared to sham, $p < 0.05$. However, the reverse
polarity group did not differ significantly with respect
to either positive treatment or sham groups. The
experiment was repeated with a further 12 rats. In the
second experiment, one-way analysis of variance also
indicated a significant effect of stimulation, $F_{(2,22)}$ =
7.546, $p < 0.003$. Comparison with Tukey's HSD test
revealed a significant difference between positive and
sham groups, $p < 0.01$, and also between positive and
reverse polarity groups, $p < 0.05$. Analysis of the
combined results from both groups (n = 27) indicated a
highly significant difference between positive polarity
and sham, $p < 0.01$, and between positive polarity and
reverse polarity, $p < 0.01$. In neither of the two
polarity experiments, nor in the combined results, did
the reverse polarity group differ significantly from
sham.

The results of Experiment 5, which tested the
effect of various electrode placements, indicates a
significant effect of electrode placement, $F_{(2,33)}$ =
5.945, $p < 0.01$ by one-way analysis of variance. Post
hoc comparison using Tukey's HSD test indicates a
significant difference with the group receiving standard
electrode placement showing an increased TFL compared
with either of the monolateral stimulation groups,
$p < 0.05$.

     EXAMPLE 3: Effect of 5-HTP Administration
             and Inhibition of Enzymes
             Involved in Making Serotonin

An experiment designed to assess the effect of
concomitant adminstration of the precursor of the
neuroactive chemical promoter serotonin, 5-HTP, and

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subsensory, transcranial electrostimulation was con-
ducted as follows. Ninety-two male Simonsen Albino rats
(Simonsen Laboratories, Inc., Gilroy, CA) weighing
between 200 and 250 g at the time of testing were housed
in groups of six with food and water available ad lib
and on a 12/12 light/dark cycle. They were handled
three minutes per day for 42 days and then restrained 10
minutes for the following three days. Electrodes were
implanted as described in Example 1, and electronics
were as described in Example 2.

Fifteen minutes prior to stimulation, the rats were
placed in adjustable cylindrical plastic restrainers.
Leads were connected to the electrodes with the positive
lead (that delivering a positive first phase) attached
the animals' right ears. Each experiment included a
sham control group consisting of rats which were
implanted, restrained and connected as above, but which
did not receive any electrical stimulation.

All observations were carried out by operators who
were blind to the stimulus parameters and to the
identity of animals receiving stimulation. Care was
taken to exclude systematic influences on the data
arising from stimulation at different times of day by
stimulating and testing equal numbers of animals from
each stimulus or sham group in every stimulation
session.

The rats were pretreated with dl-p-chloropheny-
lalanine, methyl ester (pCPA), Sigma Chemical Co. (300
mg/kg i.p.) dissolved in 2.0 ml of saline or with saline
vehicle 48 hours prior to testing, and were also treated
with either 5-hydroxy-dl-tryptophan, ethyl ester, (5HTP)
Sigma Chemical Co. (100 mg/kg i.p.) dissolved in 1.5 ml.
of saline in a 37°C water bath or with saline vehicle 30
minutes prior to testing. At that time the rats were
placed in the cylindrical plastic restrainers and the
elctrodes attached as described above. They then
received 30 minutes of either TE or sham TE treatment
(Table I).

Treatment Groups:
TABLE I. Rats were assigned to the following

	TE			SHAM	
GROUP	TREATMENT		GROUP	TREATMENT	
A	pCPA + 5-HTP		E	pCPA + 5-HTP	
B	pCPA + Saline		F	pCPA + Saline	
C	Saline + 5-HTP		G	Saline + 5-HTP	
D	Saline + Saline		H	Saline + Saline	

Analgesia was assessed using a pressure technique modified from that described by Randall, L.O. and J.J. Selitto, III Arch. Int. Pharmocodyn. 409-419 (1957). Each rat's tail was subjected to pressure (1 inch from the tip) exerted by a metal wedge mounted on the end of a syringe which was pneumatically driven. The amount of pressure withstood by the rat was read from a mercury manometer as the rat made the first coordinated motor response to remove its tail. One experimenter operated the wedge and cut off air pressure when the rat responded as the other experimenters read the height reached by the mercury on the manometer. This pressure reading averaged over four trials was taken as the mean tolerated peak pressure (TPP). The difference in mean TPP, before and after TE, was taken as a measure of analgesia. Pressures values are expressed as mm of mercury (Hg) ± standard error.

TE treatment resulted in increased tolerated peak tail pressure. Considering all TE treated groups (Groups A - D, Table I), the average tolerated peak tail pressure was 18.2 mm Hg or 613 percent higher than in the sham treated groups collectively ($p < .001$, Figs. 1,2). The saline-TE treated group (Group D, Table I) tolerated an average of 29 mm Hg more peak pressure than did the saline-sham TE group (Group H, Table I). Treatment with pCPA diminished TE induced analgesia.

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manometer as the rat made the first coordinated motor
response to remove its tail. One experimenter operated
the wedge and cut off air pressure when the rat
responded as the other experimenters read the height
reached by the mercury on the manometer. This pressure
reading averaged over four trials was taken as the mean
tolerated peak pressure (TPP). The difference in mean
TPP, before and after TE, was taken as a measure of
analgesia. Pressures values are expressed as mm of
mercury (Hg) ± standard error.

TE treatment resulted in increased tolerated peak
tail pressure. Considering all TE treated groups
(Groups A - D, Table I), the average tolerated peak tail
pressure was 18.2 mm Hg or 613 percent higher than in
the sham treated groups collectively (p < .001, Figs.
1,2). The saline-TE treated group (Group D, Table I)
tolerated an average of 29 mm Hg more peak pressure than
did the saline-sham TE group (Group H, Table I).
Treatment with pCPA diminished TE induced analgesia.

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While saline-TE treated rats (Group D, Table I) toler-
ated an average of 29.3 ± 4.0 mm Hg, pCPA-TE treated
rats (Group B, Table I) tolerated only 2.5 ± 1.0 mm Hg,
a decrease of 91.5 percent (p < .001, Fig. 1).

Further treatment of pCPA treated rats with 5HTP
resulted in saline control values of TE induced
analgesia (Fig. 2). Neither the values of the pCPA-5HTP
treated rats (25.4 ± 1.7 mm Hg; Group A, Table I) nor
those of the saline-5HTP treated rats (29.8 ± 2.1; Group
C, Table I) differed significantly from the TPP values
of the saline-TE treated rats (29.3 ± 4.0; Group D,
Table I). Among the sham treated groups, the pCPA-
saline (Group F, Table I) and pCPA-5HTP (Group E, Table
I) group values were low compared with the saline-saline
(Group H, Table I) group values, but the difference fell
short of statistical significance.

EXAMPLE 4: Combined Effects of Transcranial
Electrostimulation and i.c.v. Thiorphan

An experiment was conducted in which the combined
effect of TE and the enkephalinase inhibitor thiorphan
was assessed on analgesia. The subjects were 20 male
Sprague-Dawley rats, weighing 200-225 grams. Subjects
were maintained on ad lib food and water and a 12 hour
light/dark cycle. Seven days prior to the experiment,
each rat was placed under Innovar (tm) anesthesia,
implanted with gold-plated stainless steel electrodes
through the apex of the anti-helix of each pinna as
described in Example 2, and stereotaxically cannulated
in the third ventricle. Each cannula placement was
subsequently confirmed by dye injection and histological
examination. On the two days preceding the experiment,
each rat was habituated to a cylindrical plastic
restrainer.

Each rat was placed in the restrainer and pretested
for nociceptive sensitivity by measurement of tail flick
latency on the 50°C wet tail flick test (average of
three trials). The ear electrodes of each rat were then

connected to the stimulator through leads with a 200
K ohm resistor in series. Ten rats then received 30
minutes of TE stimulation (10 Hz, 10 μA, 2 msec pulse
width), while ten rats (the "sham stimulation group")
received no stimulation.

Beginning concurrently with TE or sham stimulation,
five rats from each group then received 250 μg thiorphan
i.c.v. (Peninsula Laboratories) in saline with 3%
ethanol. A motor driven syringe delivered a gradual
infusion of 5 μl/min of 2.5 mg/ml thiorphan solution
over a 20 minute period. Five rats from each group were
infused only with the injection vehicle of 3% ethanol in
saline. Immediately following drug and electro-
stimulation treatment, all rats were retested under
"blind" conditions for tail flick latency. Each
animal's analgesia score was the percentage increase in
tail flick latency from pretest to posttest. These
scores were analyzed by two-way analysis of variance.
The group receiving combined thiorphan and TE treatment
was compared with all other groups according to
Dunnett's Procedure for post-hoc comparison of a single
treatment group with all others.

The results are shown in Fig. 3. ANOVA indicated a
significant electrostimulation effect (TE vs. sham),
$F(1,16) = 9.29$, $p < .01$, and a significant drug effect
(thiorphan vs. vehicle), $F(1,16) = 29.54$, $p < .01$. The
group receiving both TE and thiorphan had a signifi-
cantly greater percentage increase in tail flick latency
than any of the other groups, $p < .01$, according to
Dunnett's Procedure.

EXAMPLE 5: Combined Effects of TE and
i.p. Acetorphan - Analgesia

An experiment similar to that described in Example
4 was conducted with the systemically active
enkephalinase inhibitor acetorphan. The subjects were
84 male Sprague-Dawley rats weighing 180-220 grams.
Each rat was implanted with ear electrodes under halo-

thane anesthesia and subsequently habituated and pre-
tested for tail flick latency as in Example 4. Forty-
two rats then received i.p. injections of 15 mg/kg
acetorphan (donated by J.C. Schwartz, INSERM, Paris) in
a vehicle of ethanol (10%)/cremophor EL (10%)/saline
(80%). Forty-two rats received the injection vehicle
alone. Beginning five minutes after injection, half of
the acetorphan recipients and half of the vehicle
recipients received 30 min. of TE as in Example 4. The
remaining half of each group received 30 min. of sham
stimulation only. All rats were then immediately
retested under "blind" conditions for tail flick latency
and their analgesia scores were expressed as percentage
increase from pretest to posttest latencies. These
scores were analyzed by the same procedures employed in
Example 4.

The results are shown in Fig. 4. ANOVA indicated a
significant electrostimulation effect (TE vs. sham),
$F(1,80) = 13.75$, $p < .01$, and a significant drug effect
(acetorphan vs. vehicle), $F(1,80) = 31.93$, $p < .01$. The
group receiving both TE and acetorphan had a signifi-
cantly greater percentage increase in tail flick latency
than any of the other groups, $p < .01$, according to
Dunnett's Procedure.

EXAMPLE 6: Combined Effect of TE and
L-tryptophan - Analgesia

Another experiment was conducted in which the
neuroactive chemical promoter L-tryptophan, the amino
acid precursor of serotonin, was administered con-
comitantly with transcranial electrostimulation as
follows. The subjects were 40 male Sprague-Dawley rats,
weighing 200-250 grams. Subjects were maintained on ad
lib food and water and a 12 hour light/dark cycle. Four
days prior to the experiment, each rat was placed under
halothane anesthesia and implanted with gold-plated
stainless steel electrodes through the apex of the
anti-helix of each pinna as described in Example 2. On

the three days preceding the experiment, each rat was
habituated to a cylindrical plastic restrainer.

Each was placed in a restrainer and pretested for
nociceptive sensitivity by measurement of tail flick
latency on the 50°C wet tail flick test (average of
three trials). Each rat was then injected i.p. either
with 200 mg/kg L-tryptophan in a vehicle of isotonic
saline (95%)/2N HCL (5%) or with vehicle alone. This
dose was selected on the basis of small pilot
experiments. Beginning 40 minutes after injection, each
rat was placed again in a restrainer, and the ear
electrodes of each rat were connected to the stimulator
through leads with a 200 K ohm resistor in series.
Twenty rats then received 30 minutes of TE stimulation
(10 Hz, 10 µA, 2 msec pulse width), while twenty rats
(the "sham stimulation group") received no stimulation.
A 2x2 factorial design was employed so that ten rats
received L-tryptophan and TE, ten rats received
L-tryptophan and sham stimulation, ten rats received
vehicle only and TE and ten rats received vehicle only
and sham stimulation.

Immediately following TE or sham stimulation, all
rats were retested under "blind" conditions for tail
flick latency. Each animal's analgesia score was the
percentage increase in tail flick latency from pretest
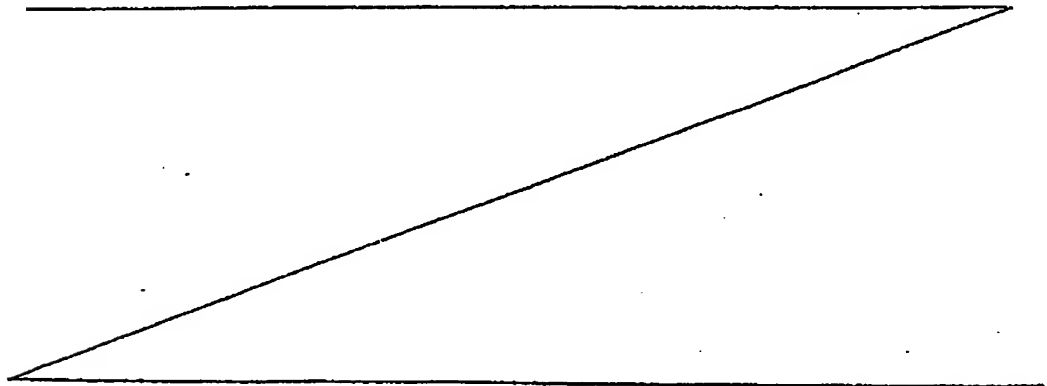to posttest. The results are set out in Table 2.

TABLE 2

COMBINED AND SEPARATE ANALGESIC EFFECTS OF TE AND L-TRYPTOPHAN: ANALGESIA SCORES (PER CENT CHANGE PRETEST TO POSTTEST) (MEAN ± SEM)

	TE	SHAM STIMULATION
L-TRYP i.p.	65.4 ± 16.6 *	-2.1 ± 4.2
VEHICLE i.p.	33.2 ± 9.8	-5.0 ± 7.5

*p < .05 vs. all other groups (Dunnett's Test)

Two-way analysis of variance indicated a significant electrostimulation effect (TE vs. sham), $F(1,36) = 25.42$, $p < .01$, and a significant drug effect (L-tryptophan vs. vehicle), $F(1,36) = 3.91$, $p < .05$. The group receiving both TE and L-tryptophan had a significantly greater percentage increase in tail flick latency than any of the other groups, $p < .05$, according to Dunnett's Procedure for post-hoc comparison of a single treatment group with all others.

EXAMPLE 7: Combined Effects of TE with D-phenyl-
alanine and Tyrosine - Analgesia

The experiment described in Example 6 is repeated, substituting the amino acid D-phenylalanine or the amino acid D-tyrosine for the neuroactive chemical promoter L-tryptophan. A dosage of about 100 to about 500 mg of the amino acid per kg rat weight i.p. in saline is employed.

EXAMPLE 8: Combined Effects of TE with
L-tryptophan and D-phenylalanine
- Morphine Abstinence Syndrome

An experiment in which the efficacy of the neuroactive chemical promoters D-phenylalanine and L-tryptophan in ameliorating the withdrawal symptoms of morphine addiction is conducted as follows. Rats are maintained as set out in Example 1 and electrodes are

implanted and electronics set up as described in Example
2. However, while under anesthesia, the rats are
implanted subcutaneously in the scapular region with
Alzet (tm) osmotic minipumps filled with 20 mg/ml
morphine sulfate dissolved in saline or with saline
vehicle alone. The morphine-treated rats are rendered
dependent by continuous infusion of 0.93 mg/kg/hr
morphine sulfate for seven days. Abrupt abstinence is
induced by removing the pumps under halothane
anesthesia.

Twenty-four hours after pump removal, each rat
receives 30 minutes of either sham stimulation as
described in Example 1, electrostimulation as described
in Example 2, or either L-tryptophan at the dosage level
set out in Example 6 or D-phenylalanine at the dosage
level set out in Example 7. Immediately after those
treatments, the rats are placed in a clear plastic
observation chamber with a grid floor and observed for
15 minutes under blind conditions on a standard
checklist of opiate abstinence signs (wet dog shakes,
genital licks, hind foot scratches, abdominal writhes,
ptosis, dyspnea, diarrhea, and teeth chattering) as
primarily based on Gianutsos, G., et al., "The Narcotic
Withdrawal Syndrome in the Rat", in S. Ehrenpreis and A.
Neidle (Eds.), Methods in Narcotic Research, New York:
Marcel Dekker, pp. 293-310 (1975).

EXAMPLE 9: Combined Effects of TE and Acetorphan -
Morphine Abstinence Syndrome

The experiment described in Example 8 is repeated
either substituting the enkephalinase inhibitor acetor-
phan for the neuroactive chemical promoters L-tryptophan
and D-phenylalanine or administering acetorphan in
addition to those two amino acids. The same dosage
regimen and vehicle described in Example 5 is employed.

EXAMPLE 10: Combined Effects of TE and
Kelatorphin - Analgesia

The experiment described in Example 5 is repeated substituting the enkephalinase inhibitor kelatorphin for the neuroactive chemical promoter acetorphan. Kelatorphin is effective when given orally, consequently, the only change in the method set out in Example 5 is that the neuroactive chemical promoter is given orally in a suitable vehicle at a dosage of between approximately 25 and approximately 200 mg/kg body weight.

EXAMPLE 11: Separate and Combined Effects of
Transcranial Electrostimulation and
Acetorphan with L-tryptophan,
D-phenylalanine and B Complex
Vitamins

Analgesia can also be efficaciously induced with combinations of one or more neuractive chemical promoters and in combination with other substances. Using the protocols set out in Examples 3-5, humans are tested for the analgesic effect of such combinations as acetorphin and L-tryptophan, acetorphan and D-phenylalanine, acetorphan with L-tryptophan and a B vitamin such as niacinamide or vitamin B_6, acetorphan with D-phenylalanine and a B vitamin such as niacinamide or B_{12} and so on, all concommitantly with TE. Dosage levels of the neuractive chemical promoters are as follows:

L-tryptophan	about 50 mg, twice a day for a person weighing 75 kg
D-phenylalanine	between about 25 mg and about 125 mg given in two approximately equal doses per day for a person weighing 75 kg
acetorphan	about 1.6 mg per day for a person weighing 75 kg.

Dosage levels of B vitamin(s) are on the order of 10 - 20 mg/kg body weight.

EXAMPLE 12: Combined Effects of Extended TE
            with Proglumide - Morphine
            Abstinence Syndrome

An experiment was performed to determine the
separate and combined effects of TE and proglumide in
rats addicted to morphine. Proglumide is an antagonist
of the peptide neurohormone, cholecystokinin (CCK). CCK
has been implicated as a contributing factor to narcotic
tolerance and dependence. As an endogenous opioid
antagonist, CCK might be expected to interfere with the
actions of endorphins released by TE. Therefore,
proglumide, by antagonizing CCK, might be expected to
potentiate the effects of TE.

Thirty-two rats were rendered dependent by seven
days subcutaneous infusion of 0.93 mg/kg/hr morphine
sulfate via Alzet (tm) osmotic minipump as described in
Example 9. The pumps were removed under light halothane
anesthesia, initiating drug withdrawal. Twenty-four
hours after pump removal, each animal was injected i.p.
with either 10 mg/kg proglumide in saline or with saline
alone and was given TE stimulation (10 Hz, 10 μA, 30
min.) or sham treatment as described in Example 2. Thus
there were four groups: TE and proglumide, TE and
saline, sham stimulation and proglumide, and sham
stimulation alone. Each rat was then observed for 15
minutes on a standard checklist of opiate abstinence
signs (wet-dog shakes, writhes, teeth chatter, ptosis
etc.), again as described in Example 9.

The results are shown in Fig. 5. The group
receiving both TE and proglumide (extreme right on the
graph) had significantly fewer abstinence signs than any
other group according to Dunnett's test for multiple
comparisons with a single group.

EXAMPLE 13: Combined Effects of Extended TE
            with Proglumide - Morphine
            Abstinence Syndrome

The experiment described in Example 12 may be
extended wherein the drug-TE combination is given

repeatedly at regular intervals over a 24 hour period.
TE is given every three hours with the same parameters
as set out in Example 12, and proglumide is administered
as described in Example 12 just prior to each TE
administration. The drug-TE combination is expected to
produce fewer abstinence signs in the experimental
subjects than would proglumide or TE given alone.

EXAMPLE 14: Combined Effects of TE and Pyrido
[2,3-e]-as-Triazin Derivatives and
Pharmaceutical Preparations

Analgesia, sedation, narcosis potentiation,
tetrabenzine antagonism and anti-phlogistic effects can
be efficaciously induced with combined administration of
TE as set out in examples 3-6 and
pyrido[2,3-e]-as-triazine (PAT) derivatives of the
following general formula wherein:
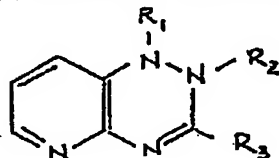
R^1 and R^2 each stand for a C_{1-20} alkylcarbonyl,
halogenated $(C_{1-4}$ alkyl)-carbonyl,
C_{1-4} alkoxycarbonyl, benzoyl, phenyl-$(C_{1-4}$
alkyl)-carbonyl or phenyl-$(C_{2-4}$ alkenyl)-
carbonyl group or a 5-10-membered mono- or
bicyclic nitrogen-containing heterocyclic acid
residue (preferably a pyridylcarbonyl group)
containing optionally one or more additional
nitrogen, oxygen and/or sulfur atoms in the
heterocyclic ring, and optionally one or more
identical or different substituents selected
from the group consisting of halogen, C_{1-4}
alkoxy, nitro and hydroxy are attached to the
aromatic or heterocyclic rings, furthermore
one of R^1 and R^2 may also stand for hydrogen
atom, or

R^1 and R^2 may form, together with the adjacent
    nitrogen atoms, a pyrazole-2,4 ring having
   optionally a C_{1-6} alkyl substituent in
    position 3, and

R^3 stands for hydrogen, halogen, C_{1-4} alkoxy,
    amino, mono-(C_{1-6} alkyl)-amino, di-(C_{1-6}
    alkyl)-amino, hydroxy, alkylated or acylated
    hydroxy, morpholino, peperazino, N-(C_{1-6}
    alkyl)-piperazino, N-benzylpiperazino or
    N-pyridylpiperazino group.

Pharaceutically acceptable acid addition salts of PAT
are prepared by acylating the respective 1,2
unsubstituted 1,2 dihydro-PAT derivatives. These
substances are described in U.S. Patent 4,324,786,
incorporated herein by this specific reference to that
patent.

The pharmaceutical preparations are for enteral or
parental administration to warm-blooded animal(s) and
contain the pharmacologically active ingredient alone or
together with a pharmaceutically acceptable carrier.
The dosage of the active ingredient depends on age,
individual condition, method of administration and body
weight. In normal patients, the estimated approximate
dose in the case of oral administration is from 200 to
500 mg/kg of body weight.

EXAMPLE 15: Combined Effects of TE and
            Heteramino Benzofurans

Analgesia and anti-inflammatory effects can be
efficaciously induced with combined administration of TE
as set out in Examples 3-6 and heteramino benzofuran
derivatives of the general formula
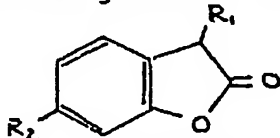
in which R_1 represents hydrogen or an aliphatic
    radical, R_2 represents an amino group
    di-substituted by a divalent hydrocarbon

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radical, and the aromatic ring A may be
additionally substituted, and their salts
and/or isomers.

These substances are described in U.S. Patent 4,451,462,
which patent is incorporated herein by this specific
reference thereto.

The pharmaceutical preparations are for enteral or
parental administration to warm-blooded animal(s) and
contain the pharmacologically active ingredient alone or
together with a pharmaceutically acceptable carrier.
The dosage of the active ingredient depends on age,
individual condition, method of administration and body
weight. In normal humans, the estimated approximate
dose in the case of oral administration is from 2 to 10
mg/kg of body weignt.

EXAMPLE 16: Combined Effects of TE
                        and Isochromans

Antipsychotic and hypotensive effects can be
efficaciously induced with combined administration of TE
and amino derivatives of isochromans of the general
formula given in U.S. Patent 4,487,774, incorporated
herein by this specific reference to that patent.

The pharmaceutical preparations are for enteral or
parental administration to warm-blooded animal(s) and
contain the pharmacologically active ingredient alone or
together with a pharmaceutically acceptable carrier.
The dosage of the active ingredient depends on age,
individual condition, method of administration and body
weight. In normal humans, the estimated approximate
dose in the case of oral administration is from 2 to 10
mg/kg of body weigh.

EXAMPLE 17: Combined Effects of TE with
                        L-Tryptophan and Acetorphan -
                        Analgesia

The combined effects of administration of TE
together with both the amino acid L-tryptophan and the
enkephalinase inhibitor acetorphan are assessed as
follows. Rats are maintained in accordance with the

protocols set out in Example 3, and tested for the
combined effects of TE and these neuroactive chemical
promoters using the tail flick latency model described
in that same example. TE is given as set out in Example
4 and acetorphan dosages are the same as set out in
Example 4. In addition, L-tryptophan is administered to
the rats in the dosages described in Example 5. It is
expected that the results seen in both Examples 4 and 5
as described above will be even more dramatic when both
neuroactive chemical promoters are administered
concurrently with TE.

EXAMPLE 18: Effect of TE on Analgesia

The 80 subjects participating in this study all had
intractable low back pain. This type of pain limits
musculo-skeletal output, and is therefore subject to a
more objective measurement of the analgesic measures
which may be applied. An isokinetic, automated exercise
device (Kin-Com^{tm}) was used to test the subjects'
ability to output musculo-skeletal torque before and
after administration of TE. Because of the varying
nature of each subject's pain, the ability to trigger
pain in each subject, each subject's idiosyncratic use
of drugs, and each subject's differing physical
condition, an experimental design was implemented in
which each subject served as his own control.

Volunteering subjects were randomized into two
matched groups. Each subject participated in a 2 x 2
cross-over design in which he received two randomly
assigned analgesic treatments and two sham treatments
(placebos). The analgesic treatment was application of
low current, transcranial electrostimulation therapy
(TE).

Subjects were tested on the Kin-Com every fourth
day. The tests were performed at two different angular
velocities, 15 and 30 degrees/sec. Each subject was
challenged in eccentric (muscle relaxing) and concentric
(muscles contracting) modes. These tests were conducted
pre- and post-TE. Results showed significant

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differences between treatment days and significant differences in two of four days between treatment and sham administration of TE.

EXAMPLE 19:   Effect of TE to Relieve Nicotine
               Withdrawal Systoms - Smoking
               Cessation

Transcranial electrostimulation (TE) has been applied in double blind clinical trails to long term smokers (at least one pack per day) who indicated they wished to quit smoking. Three different portocols were investigated--one treatment per day for eight days, two treatments per day for five days, or one treatment per day combined with a behavior modification program. In the first protocol, treatments were given Monday through Friday and then the following Monday through Wednesday. In the second protocol, treatments were delivered Monday through Friday. The behavior modification program used in the third protocol was applied in the recognition that TE served to assist in overcoming short term withdrawal symptoms, but that psychological craving required address by behavior modification to induce long term support against recidivism.

The subjects selected for participation in the study smoked at least one pack of cigarettes per day for at least five years, were between the ages of 18 and 55, were not pregnant, had no known threatening medical conditions, had tested negative for cannaboids and other confounding drugs. At a pre-study orientation, the subjects supplied information on the base cigarette count and brand of cigarette smoked and were prospectively randomized into two groups--treatment and placebo.

TE consisted of low current (less than 40 μA), pulsed sugnals delivered through electrodes attached to the earlobes. The stimulus was delivered below perception levels of the subjects. The duration of each treatment was approximately one hour.

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Subjects provided a daily record of the number of
cigarettes and the time that they smoked, and completed
questionaires to assess withdrawal symptoms. Saliva
samples were collected at the clinic from each subject
at orientation before the study began and on each day of
treatment. Cotinine levels were determined using a
monoclonal antibody enzyme linked immunosorbent assay
(ELISA).

Additionally, the Shiffman Withdrawal Scale, Analog
Craving Scale and an instrument to assess withdrawal
symptoms were administered regularly throughout the
study. Subjects provided urine samples at orientation
and on demand during the study. These samples were
assayed to detect marijuana, cocaine or other drugs
whose use could confound the analysis of study results.
Subjects whose drug test was positive were excluded from
the study.

Sixty-seven (67) subjects participated in the trial
administering one treatment per day. Of these, 40
received actual treatment and 27 received placebo (no
current). The quit rates verified by salivary cotinine
analysis were 55 percent in the treatment group and 22
percent in the placebo group.

Forty-one (41) subjects participated in the two
treatments per day trial. Twenty-five received actual
treatment and 16 received placebo. The quit rates were
68 percent and 38 percent respectively.

The subjects involved in the third protocol were
randomly assigned to one of four groups: treatment +
behavior modification, treatment only (behavior
modification was promised after the initial experimental
period), behavior modification only (treatment was
promised after the initial experimental period), and
placebo + behavior modification. The group which
received treatment + behavior modification had a
statistically significant greater quit rate than the
other three groups.

Example 20: Separate and Combined Effects of

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Transcranial Electrostimulation and
Acetorphan, with L-tryptophan,
D-phenylalanine, B complex vitamins
and Nicotinic acid

The experiment described in Example 11 can be
repeated using additional chemical promotors which
assist the synthesis, inhibit the degradation, and
facilitate uptake, distribution and metabolism of
neuroactive substances. Nicotinic acid in the form of
niacinamide (50 mg given once per day), vitamin B_6,
puridoxin (50 mg given once per day) and monoamine
oxidase inhibitors such as D-L-phenylalanine (500 mg
given once per day), L-glutamine, or
pyridoxal-5-phosphate (10 mg given once per day) assist
in producing enhanced analgesic effects.

* * * * *

Although described in terms of the above presently
preferred embodiments, it will be recognized by those
skilled in the art who have the benefit of this
disclosure that changes can be made to the dosages,
protocols, current levels, and so on as set out in those
examples without departing from the spirit and scope of
the present invention. All such changes are intended to
fall within the scope of the following claims.

What Is Claimed Is:

1. A method of medical treatment of a mammal comprising the concomitant administration of a neuroactive chemical promoter and transcranial electrostimulation to a mammal.

2. The method of claim 1 wherein the neuroactive chemical promoter is selected from the group comprising endorphins, indoleamines, catchecholamines, amino acids present in the central nervous system, amino acids capable of inducing a central nervous system response, cholinergic agents, peptides found in the central nervous system, peptides capable of inducing a central nervous system response, precursors of same, inhibitors of the enzymes active in the metabolic pathways involved in producing or degrading same, antagonists and binding competitors of same, and combinations of same.

3. The method of claim 1 wherein the neuroactive chemical promoter is an endorphin, an enzyme active in the metabolic pathway involved in making endorphins, a tryptamine derivative, an enzyme active in the metabolic pathway involved in making tryptamine derivatives, an amino acid, a peptide found in the central nervous system, or an antagonist of a peptide found in the central nervous system.

4. The method of claim 1 wherein the neuroactive chemical promoter is selected from the group consisting of proglumide, 5-hydroxy-dl-tryptophan, acetorphan, thiorphan, or L-tryptophan.

5. The method of claim 1 wherein the current of the transcranial electrostimulation is between approximately 5 and approximately 40 µA.

6. The method of claim 1 wherein the pulse width of the transcranial electrostimulation is between approximately 0.1 and approximately 8.0 msec.

7. The method of claim 1 wherein the frequencey of the transcranial electrostimulation is between approximately 5 and approximately 50 Hz.

8. The method of claim 1 wherein the duration of the transcranial electrostimulation is between approximately 10 and approximately 60 minutes.

9. The method of claim 1 wherein the neuroactive chemical promoter is selected from the group consisting of acetorphine, kelatorphin or thiorphan.

10. The method of claim 1 additionally comprising the concomitant administration of a second neuroactive chemical promoter.

11. A kit for providing medical treatment of a mammal, which kit comprises a neuroactive chemical promoter in a form suitable for administration to a mammal and means for concomitant administration of transcranial electrostimulation to the mammal.

12. The kit of claim 11 wherein the neuroactive chemical promoter is selected from the group consisting of:

endorphins, presursors of endorphins, inhibiters of the enzymes active in the metabolic pathways involved in producing and degrading endorphins, and binding competitors and antagonists of endorphins,

amino acids present in the central nervous system or capable of inducing a central nervous system response, inhibiters of the enzymes active in the metabolic pathways involved in producing and degrading such amino acids, and antagonists and binding competitors of such amino acids,

peptides present in the central nervous system or capable of inducing a central nervous system response, enzymes active in the metabolic pathways involved in producing and degrading such peptides, inhibiters of the inzymes involved in producing and

degrading such peptides, and antagonists and binding competitors of such peptides, and

indoleamines, enzymes active in the metabolic pathways involved in producing and degrading indoleamines, precursors of indoleamines, inhibitors of the enzymes involved in producing and degrading indoleamines, and antagonists and binding competitors of indoleamines.

13. The kit of claim 11 wherein the neuroactive chemical promoter is proglumide, 5-hydroxy-dl-tryptophan, acetorphan, thiorphan, L-tryptophan or combinations of proglumide, 5-hydroxyl-dl-tryptophan, acetorphan, thiorphan, and L-tryptophan.

14. The method of using a neuroactive chemical promoter for the manufacture of a medicament for administration to a mammal to which transcranial electrostimulation is concomitantly administered.

15. The method of claim 13 wherein the neuroactive chemical promoter is selected from the group comprising endorphins, indoleamines, catchecholamines, amino acids present in the central nervous system, amino acids capable of inducing a central nervous system response, cholinergic agents, peptides found in the central nervous system, peptides capable of inducing a central nervous system response, precursors of same, inhibitors of the enzymes active in the metabolic pathways involved in producing or degrading same, antagonists and binding competitors of same, and combinations of same.

16. A pharmaceutical composition for providing medical treatment of a mammal in combination with transcranial electrostimulation, comprising as an active ingredient a neuroactive chemical promoter.

17. The composition of claim 15 wherein the neuroactive chemical promoter is selected from the group comprising endorphins, indoleamines, catchecholamines, amino acids present in the central nervous system, amino acids capable of inducing a central nervous system

response, cholinergic agents, peptides found in the
central nervous system, peptides capable of inducing a
central nervous system response, precursors of same,
inhibitors of the enzymes active in the metabolic
pathways involved in producing or degrading same,
antagonists and binding competitors of same, and
combinations of same.

AMENDED CLAIMS
[received by the International Bureau
on 14 January 1991 (14.01.91);
original claims 2,3,4,7,9,12,13,15 and 17 amended;
new claims 18-20 added; other claims unchanged (4 pages)]

1.   A method of medical treatment of a mammal com-
prising the concomitant administration of a neuroactive
chemical promoter and transcranial electrostimulation to a
mammal.

2.   The method of claim 1 wherein the neuroactive
chemical promoter is an indoleamine, catchecholamine, amino
acid present in the central nervous system, amino acid
capable of inducing a central nervous system response,
cholinergic agent, peptide capable of inducing a central
nervous system response, or a precursor, antagonist, or
binding competitor of a neurotransmitter, inhibitor of an
enzyme active in a metabolic pathway involved in producing
or degrading same, and combinations of same.

3.   The method of claim 1 wherein the neuroactive
chemical promoter is an enzyme active in the metabolic
pathway of peptidyl opiates, a tryptamine derivative, an
enzyme active in the metabolic pathway of tryptamine
derivatives, an amino acid, a peptide which is a modulator
of the central nervous system, or a precursor, binding
competitor, or antagonist of a peptide which is a modulator
of the central nervous system.

4.   The method of claim 1 wherein the neuroactive
chemical promoter is selected from the group consisting of
proglumide, 5-hydroxy-dl-tryptophan, acetorphan, thiorphan,
or L-tryptophan.

5.   The method of claim 1 wherein the current of the
transcranial electrostimulation is between approximately 5
and approximately 40 μA.

6.   The method of claim 1 wherein the pulse width of
the transcranial electrostimulation is between approximate-
ly 0.1 and approximately 8.0 msec.

7.   The method of claim 1 wherein the frequency of
the transcranial electrostimulation is between approximate-
ly 5 and approximately 50 Hz.

8.   The method of claim 1 wherein the duration of the transcranial electrostimulation is between approximately 10 and approximately 60 minutes.

9.   The method of claim 1 wherein the neuroactive chemical promoter is selected from the group consisting of acetorphan, kelatorphin or thiorphan.

10.   The method of claim 1 additionally comprising the concomitant administration of a second neuroactive chemical promoter.

11.   A kit for providing medical treatment of a mammal, which kit comprises a neuroactive chemical promoter in a form suitable for administration to a mammal and means for concomitant administration of transcranial electrostimulation to the mammal.

12.   A kit according to claim 11, wherein the neuroactive chemical promoter is selected from the group consisting of:

precursors of endorphins, inhibitors of an enzyme active in a metabolic pathway involved in producing or degrading endorphins, and binding competitors and antagonists of endorphins,

amino acids present in the central nervous system or capable of inducing a central nervous system response, inhibitors of an enzyme active in a metabolic pathway involved in producing or degrading such amino acids, and antagonists and binding competitors of such amino acids,

peptides capable of inducing a central nervous system response, enzymes active in a metabolic pathway involved in producing or degrading such peptides, inhibitors of an enzyme involved in producing or degrading such peptides, and antagonists and binding competitors of such peptides, and

indoleamines, enzymes active in a metabolic pathway involved in producing or degrading indoleamines, precursors of indoleamines, inhibitors of an enzyme involved in producing or degrading

indoleamines, and antagonists and binding competitors of indoleamines.

13. A kit according to claim 11, wherein the neuroactive chemical promoter is proglumide, 5-hydroxy-dl-tryptophan, acetorphan, thiorphan, L-tryptophan or combinations of proglumide, 5-hydroxyl-dl-tryptophan, acetorphan, thiorphan, and L-tryptophan.

14. The use of a neuroactive chemical promoter for the manufacture of a medicament for medical treatment of a mammal with concomitant administration of transcranial electrostimulation.

15. Use according to claim 14, wherein the neuroactive chemical promoter is selected from the group comprising indoleamines, catchecholamines, amino acids present in the central nervous system, amino acids capable of inducing a central nervous system response, cholinergic agents, peptides capable of inducing a central nervous system response, or precursors, antagonists, or binding competitors of a neurotransmitter, or inhibitors of an enzyme active in a metabolic pathway involved in producing or degrading same, and combinations of same.

16. A pharmaceutical composition for providing medical treatment of a mammal in combination with transcranial electrostimulation, comprising as an active ingredient a neuroactive chemical promoter.

17. A composition according to claim 16, wherein the neuroactive chemical promoter is selected from the group comprising indoleamines, catchecholamines, amino acids present in the central nervous system, amino acids capable of inducing a central nervous system response, cholinergic agents, peptides capable of inducing a central nervous system response, or precursors, antagonists, or binding competitors of a neurotransmitter or inhibitors of an enzyme active in a metabolic pathway involved in producing or degrading same, and combinations of same.

18. A kit for providing medical treatment of a mammal, which kit comprises a neuroactive chemical promoter

in a form suitable for administration to a mammal and means
for concomitant transcranial administration of charge-
balanced, biphasic electrical current having an amplitude
of from about 5 to about 40 µA, a pulse width of from about
0.1 to about 8.0 msec, and a frequency of from about 5 to
about 50 Hz.

19. A kit according to claim 18, wherein the neuroac-
tive chemical promoter is selected from the group consist-
ing of:

     precursors of endorphins, inhibitors of an enzyme
active in a metabolic pathway involved in producing or
degrading endorphins, and binding competitors and
antagonists of endorphins,

     amino acids present in the central nervous system
or capable of inducing a central nervous system
response, inhibitors of an enzyme active in a metabol-
ic pathway involved in producing or degrading such
amino acids, and antagonists and binding competitors
of such amino acids,

     peptides capable of inducing a central nervous
system response, enzymes active in a metabolic pathway
involved in producing or degrading such peptides,
inhibitors of an enzyme involved in producing or
degrading such peptides, and antagonists and binding
competitors of such peptides, and

     indoleamines, enzymes active in a metabolic
pathway involved in producing or degrading
indoleamines, precursors of indoleamines, inhibitors
of an enzyme involved in producing or degrading
indoleamines, and antagonists and binding competitors
of indoleamines.

20. A kit according to claim 18, wherein said kit
additionally comprises a second neuroactive chemical
promoter in a form suitable for administration to a mammal.

STATEMENT UNDER ARTICLE 19

The amendments to claims 12, 15, and 17 (claims 1-10 are not addressed herein because they were not searched) are made as a result of the Applicants' discovery that the claims, as filed, listed certain substances as falling within the scope of the defined phrase "neuroactive chemical promoter" recited in independent claims 11, 14, and 16 (on which claims 12, 15, and 17 are dependent, respectively) which are ineffective for the intended purpose. Specifically, by including the peptidyl opiates referred to as endorphins in claims 12, 15, and 17, those claims were over-inclusive. The over-inclusiveness is because (1) endorphins are so rapidly digested by normal action of the enzymes present in mammals as to be rendered ineffective as a neuroactive chemical promoter if given orally and (2) techniques available for injection of endorphins into the central nervous system to avoid the action of such diges-tive enzymes are so invasive as to effectively eliminate this mode of administration as a practical method of treatment. Consequently, all references to these peptidyl opiates have been eliminated from the claims. Coinciden-tally, this amendment helps to further define over the disclosure of the Capel patent cited in the International Search Report.

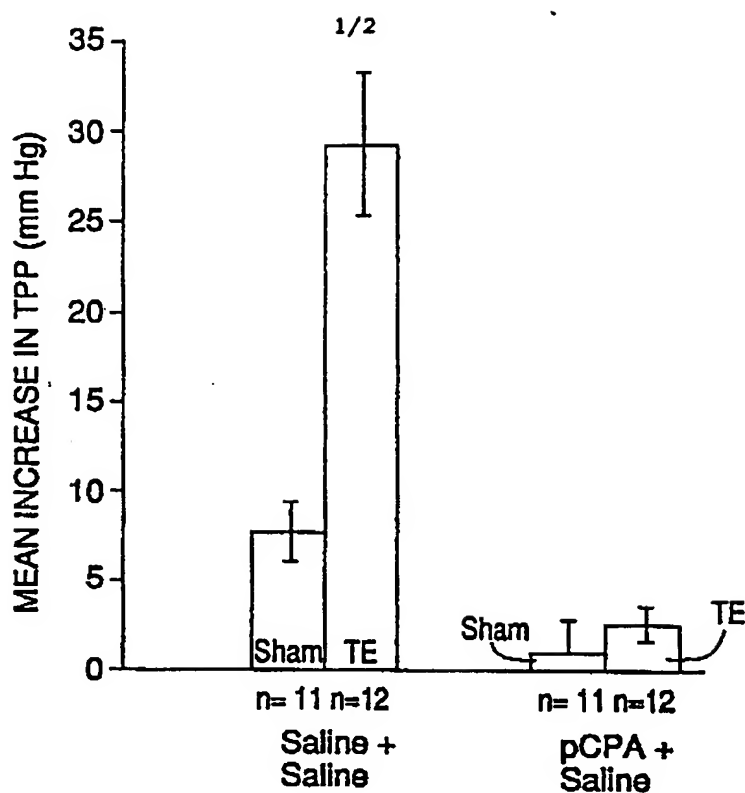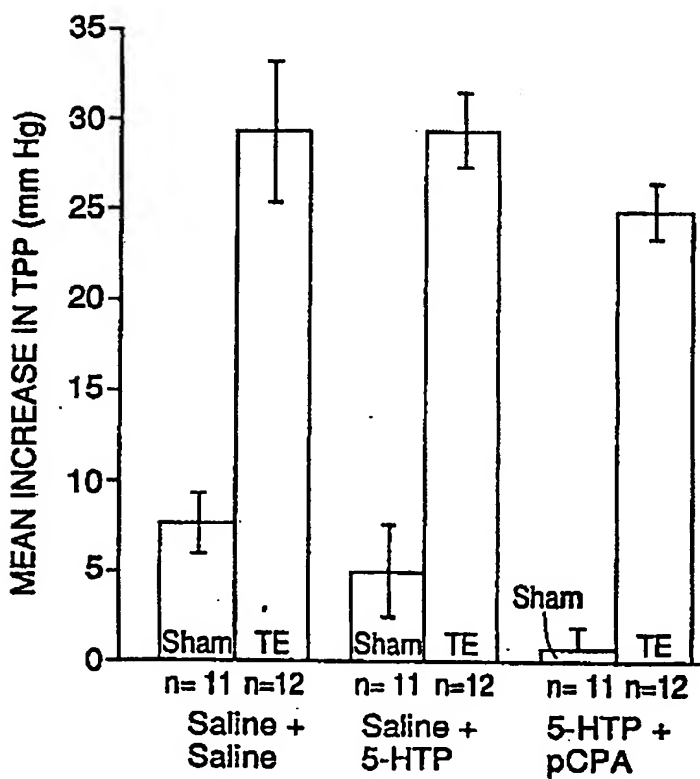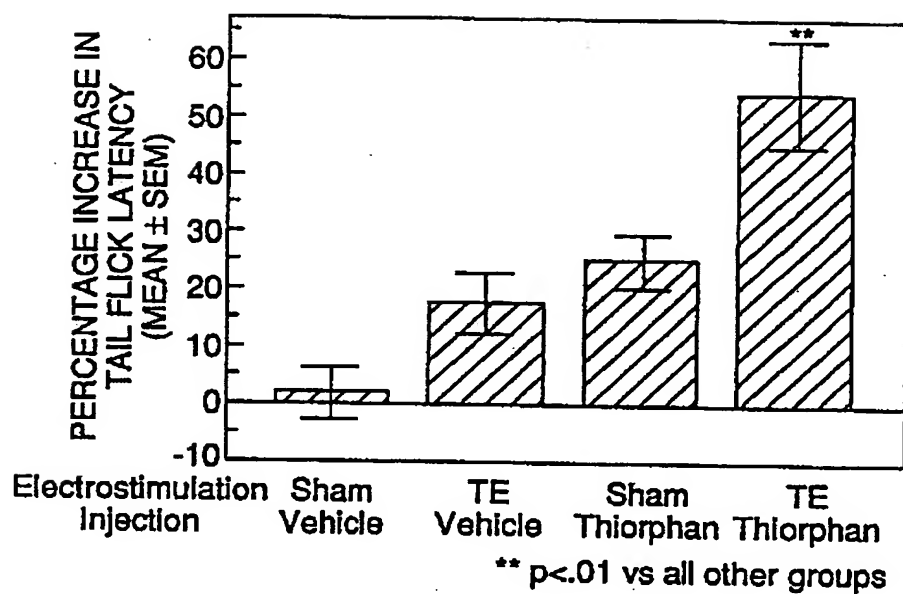Entry of the attached replacement sheets is respect-fully requested.

1/2

Fig. 1

Fig. 2

Fig. 3

INTERNATIONAL SEARCH REPORT

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) *

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC⁵: A 61 K 45/08, A 61 N 1/32

II. FIELDS SEARCHED

Minimum Documentation Searched ⁷

Classification System	Classification Symbols
IPC⁵	A 61 K

Documentation Searched other than Minimum Documentation
to the Extent that such Documents are Included in the Fields Searched ⁸

III. DOCUMENTS CONSIDERED TO BE RELEVANT ⁹

Category *	Citation of Document, ¹¹ with Indication, where appropriate, of the relevant passages ¹²	Relevant to Claim No. ¹³
X	Chemical Abstracts, volume 110, no. 23, 5 June 1989, (Columbus, Ohio, US), D.H. Malin et al.: "Augmented analgesic effects of enkephalinase inhibitors combined with transcranial electrostimulation", see page 64, abstract 205560u & Life Sci. 1989, 44(19), 1371-6 (Eng).	11-17
P,X	Chemical Abstracts, volume 113, no. 13, (Columbus, Ohio, US), D.H. Malin et al.: "Augmented analgesic effects of L-tryptophan combined with low current transcranial electrostimulation", see page 73, abstract 10923k & Life Sci. 1990, 47(4), 263-7 (Eng).	11-17

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* Special categories of cited documents: ¹⁰

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
23rd October 1990	14. 11. 90

International Searching Authority	Signature of Authorized Officer
EUROPEAN PATENT OFFICE	M. PEIS

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

| A | US, A, 4646744 (IFOR D. CAPEL) 3 March 1987 see column 7, lines 24-38 (cited in the application) | 11-17 |

V.☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE ¹

This International search report has not been established in respect of certain claims under Article 17(2).(a) for the following reasons:

1.☒ Claim numbers 1-10 because they relate to subject matter not required to be searched by this Authority, namely:

Pls. see Rule 39.1 (iv) - PCT:

Method for treatment of the human or animal body by therapy or surgery, as well as diagnostic methods.

2.☐ Claim numbers........., because they relate to parts of the International application that do not comply with the prescribed requirements to such an extent that no meaningful International search can be carried out, specifically:

3.☐ Claim numbers......... because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI.☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING ²

This International Searching Authority found multiple inventions in this International application as follows:

1.☐ As all required additional search fees were timely paid by the applicant, this International search report covers all searchable claims of the International application.

2.☐ As only some of the required additional search fees were timely paid by the applicant, this International search report covers only those claims of the International application for which fees were paid, specifically claims:

3.☐ No required additional search fees were timely paid by the applicant. Consequently, this International search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4.☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (supplemental sheet (2)) (January 1985)

ANNEX TO THE INTERNATIONAL SEARCH REPORT
ON INTERNATIONAL PATENT APPLICATION NO.   US 9004443
             SA   39474

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the European Patent Office EDP file on 05/11/90
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
US-A- 4646744	03-03-87	AU-B- AU-A- EP-A- WO-A-	571723 4603185 0238480 8700063	21-04-88 30-01-87 30-09-87 15-01-87

EPO FORM P0459

For more details about this annex : see Official Journal of the European Patent Office, No. 12/82

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